INTRODUCTION: Type 2 Diabetes (T2D) is a metabolic disease characterized by disrupted glucose control. This disease leads to persistent high blood sugar levels, which adversely affects various organ systems including the skeleton. Despite having normal to high bone mineral density, T2D patients are more prone to fracture than non-diabetics, particularly in the hip, vertebrae, and distal forearm [1]. This vulnerability is associated with elevated sugar levels, prompting the production of Advanced Glycation End Products (AGEs) such as Pentosidine (PEN) and Carboxymethyl-Lysine (CML). These are harmful compounds that alter the collagen compartment within the bone matrix and ultimately impact bone’s mechanical stability. While research has examined total fluorescent AGEs (fAGEs) [2], PEN [3], and CML [2,4] levels in bone specimens, it is not clear how they relate to each other in T2D specimens. This study addresses this gap by assessing glycation content in T2D and non-diabetic bone specimens across young and old donors. We hypothesize there will be higher fAGEs, PEN, and CML in T2D and older bone compared to non-diabetic and young bone, and that fAGEs, PEN, and CML will be positively correlated with each other.

METHODS: Cadaveric human tibias from four female donors were collected from a tissue donor bank (Anatomic Gifts Registry, Hanover, MD). The donors were T2D-33 years old (young T2D), T2D-77 years old (old T2D), non-diabetic-35 years old (young non-D), and non-diabetic-74 years old (old non-D). Twelve longitudinal cortical beams extracted from the midshaft of each donor (n=48) were cut with a diamond blade saw to obtain specimens weighing approximately 75mg. Specimens were processed to obtain bone hydrolysates, which were then used to measure total fluorescent AGEs by a fluorometric assay [2] and PEN by high performance liquid chromatography (HPLC; Shimadzu Prominence LC-2030C) equipped with a fluorescence detector (Shimadzu Prominence RF-20A) and C18 column (Shimadzu, #220-91394-00). The distal portion of the same beams were cut (100 mg) and pre-processed [2,4]. Total protein was measured by a Bradford assay and CML was quantified in the pre-processed samples by a Cell BioLabs CML Competitive ELISA kit (STA-816) following manufacturer's instructions. Kruskal-Wallis tests and Wilcoxon post-hoc were used to compare differences between groups.

RESULTS: Total fAGEs were increased in T2D compared to healthy bone (+53.83%, p≤0.05 for young donors; +35.25%, p≤0.05 for old donors). There was no significant difference in fAGEs between healthy young and healthy old groups (p=0.05). T2D young (+80%, p≤0.05), T2D old (+108, p≤0.05), and healthy old donors (+108%, p≤0.05) had significantly higher PEN compared to healthy young donors. CML levels were significantly higher in T2D old compared to healthy old (+54.14%, p≤0.05) and healthy young (+175%, p≤0.05) donors. A positive correlation was observed between fAGEs and PEN (r=0.579, p≤0.05) and between fAGEs and CML (r=0.344, p≤0.05). PEN and CML content did not correlate with each other (r=0, p>0.05). Results are illustrated in Fig 1.

DISCUSSION: In support of our hypothesis, total fAGEs, PEN, and CML were greater in T2D and older age specimens compared to their respective controls. This may be attributed to higher levels of blood glucose present, which are subsequently exposed to the collagen matrix and can result in formation of more glycation products. Specifically, fAGEs were higher in old vs young and in T2D vs non-diabetic, indicating the disease state had a greater influence on accumulation of fAGEs rather than age. PEN was higher in all groups compared to the healthy young group, indicating that both age and diabetic condition can influence the accumulation of PEN. CML levels were increased in only the T2D old group compared to healthy old and healthy young groups, while no difference was present between young donors with and without diabetes. This suggests that CML was primarily impacted by disease state. Both PEN and CML had moderate positive correlations with fAGEs, indicating that although there is a relationship between these crosslinks and fAGEs, they may not necessarily serve as standalone predictors of total fAGE content.

SIGNIFICANCE/CLINICAL RELEVANCE: Increased bone fracture risk in T2D may be due to changes in bone quality such as the accumulation of collagen crosslinks. We report for the first time a comparative report of fAGE, PEN, and CML levels in T2D vs non-diabetic bone specimens.


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![Fig 1](image-url) (A) Total fAGEs in four experimental groups, (B) Correlation of fAGEs with PEN levels, and (C) Correlation of fAGEs with CML levels. * indicates p≤0.05